Clin. exp. Immunol. (1986) **66**, 393–398

The distribution of gluten-sensitive lymphocytes in coeliac patients—is it related to dietary gluten?

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(Accepted for publication 17 June 1986)

SUMMARY

Cell-mediated immunity to gluten within the peripheral blood and jejunal mucosa of the same coeliac patients was measured simultaneously using migration inhibition tests. There was an inverse correlation in migration indices indicating that as cell-mediated immunity became more detectable in the peripheral blood, it was less so in the jejunal mucosa and vice versa. There was virtually no relationship, however, between this correlation and the gluten content of the diet in these coeliac patients.

Keywords coeliac gluten lymphocytes migration inhibition

INTRODUCTION

There is considerable evidence of humoral and cellular immunity to gluten in coeliac disease. Although it is likely that such mechanisms could produce the typical small intestinal mucosal lesion of coeliac disease as a primary event, there is no definite evidence that this is the case.

It has been proposed that cell-mediated immunity (CMI) to gluten could cause the villous atrophy found in coeliac patients (Ferguson, 1974) and evidence of CMI to gluten or gliadin within untreated coeliac mucosa has been produced (Ferguson *et al*, 1975; Howdle, Bullen & Losowsky, 1982). There is also evidence of CMI in the peripheral blood of coeliac patients, and peripheral blood leucocytes are more sensitive to gluten in treated than in untreated patients (Holmes, Asquith & Cooke, 1976; Bullen & Losowsky, 1978; Simpson *et al.*, 1981).

These findings accord with the suggestion made by Holmes *et al.* (1976) that 'a change in the distribution of sensitised cells occurs in coeliac patients depending upon the type of diet ingested, whether gluten-containing or gluten-free'. Based on this suggestion, the hypothesis would be that, in untreated coeliac patients, gluten-sensitive lymphocytes are sequestered in the gut in response to dietary gluten, whereas when gluten is excluded from the diet a higher proportion of these sensitized cells would be present in the blood.

In order to test this hypothesis, we have assessed CMI to gluten fraction III simultaneously in jejunal mucosa and peripheral blood of coeliac patients.

MATERIALS AND METHODS

Patients. There were seven adult patients with a flat jejunal mucosa on biopsy showing the histological features of total villous atrophy, crypt hyperplasia and inflammatory cell infiltration. Four of these had never had a gluten-free diet, were taking a gluten-containing diet at the time of biopsy and have subsequently shown a good clinical and morphological response to a gluten-free

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diet; they were, therefore, untreated coeliac patients. Three patients were siblings of a known coeliac patient and it was subsequently discovered that they were already taking a diet low in gluten before presenting for investigation and treatment. They were, therefore, partially treated in dietary terms, although their jejunal mucosae were initially abnormal, showing changes typical of untreated coeliac disease. On a strict gluten-free diet, there was morphological improvement to normal in all. These three patients were included in the untreated coeliac patient group.

There were nine patients who initially had a severe enteropathy and who had been on a gluten-free diet for varying lengths of time, between 6 months and 20 years. All had shown a good clinical and morphological response to gluten withdrawal, with normal or near normal jejunal histology post-treatment. These were treated coeliac patients.

Details of the treated coeliac patients are given in Table 1. Included is an assessment of the strictness of adherence to a gluten-free diet. The degree of strictness was assessed on a simple grading system of 0, 1, 2 or 3, corresponding to nil, poor, moderate or good adherence to the diet, and the assessment was made, prior to the biopsies being taken, from a personal knowledge of the coeliac patients by two independent observers.

Jejunal biopsies. These were taken, with informed consent, using a multiple hydraulic biopsy capsule, from the jejunum at the level of the duodeno-jejunal flexure. At the same time, 20 ml of peripheral venous blood were taken for immediate use in a leucocyte migration inhibition assay. These studies were approved by the local Ethical Committee.

Organ culture of jejunal biopsies. This was performed as previously described (Howdle *et al.*, 1981). The biopsies were cultured in medium, either with or without gluten fraction III (GFIII) (Frazer *et al.*, 1959) at a concentration of 1 mg/ml. The culture media were removed after 5 h of culture and, to assess whether cell mediated immune mechanisms had been operating, they were used immediately in a migration inhibition assay.

Assessment of cell mediated immunity using leucocyte migration inhibition assays. CMI in culture medium was assessed as already described (Howdle *et al.*, 1982). The presence of lymphokines (representing CMI in the jejunal mucosa) was assayed by measuring the migration of normal (non-coeliac) peripheral blood leucocytes from a healthy volunteer into culture medium recovered from organ culture of the coeliac mucosal biopsies as outlined above. Migration into culture medium which had contained GFIII during culture was compared with migration into culture medium from a second biopsy from the same patient obtained at the same time, which had been cultured for the same length of time without GFIII and then reconstituted with GFIII at the same concentration (1 mg/ml) after culture. The assays were performed in quadruplicate and the results expressed as a migration index, that is the ratio of mean area of migration into GFIII-containing culture medium divided by the mean area of migration into culture medium reconstituted with GFIII.

Table 1. Treated coeliac patients

No.	Sex	Age	Jejunal histology post-treatment	Time on gluten-free diet (years:months)	Strictness of gluten-free diet
1	F	45	Normal	1:6	3
2	M	45	Mild PVA	1:6	3
3	F	49	Normal	10:0	3
4	M	58	Normal	2:0	3
5	M	63	Mild PVA	1:0	3
6	F	62	Severe PVA	7:6	1
7	M	21	Severe PVA	20:5	1
8	M	44	Mild PVA	4:6	2
9	F	40	Mild PVA	0:6	2

PVA, Partial villous atrophy.

CMI in the peripheral blood was assayed, as described previously (Bullen & Losowsky, 1978), by measuring the migration of peripheral blood leucocytes from the coeliac patients into freshly prepared culture medium either with or without GFIII (1 mg/ml). The assays were performed in quadruplicate and the migration index was calculated as the ratio of mean area of migration into GFIII-containing medium divided by the mean area of migration into culture medium alone.

These two types of migration inhibition assay were therefore performed simultaneously using either peripheral blood leucocytes or culture medium from mucosal biopsies obtained from the same coeliac patients at the same time.

RESULTS

The results of the migration indices, in the two groups of coeliac patients, for peripheral blood leucocytes and jejunal mucosal culture media, measured simultaneously, are shown in Table 2.

There was no significant difference between the two groups of patients for indices in peripheral blood, the untreated coeliac patients showed similar migration inhibition to the treated patients. Similarly, there was no significant difference between the two groups for indices in organ culture media. The untreated patients' results were similar to those in treated patients, there being significant inhibition in only one untreated patient (No. 7). These results suggest that this group of untreated coeliac patients were behaving in a fashion similar to treated coeliac patients, as regards these two migration inhibition tests (Bullen & Losowsky, 1978; Howdle *et al.*, 1982).

From the point of view of the present experiment, however, the more important comparison to make was between migration indices in peripheral blood and jejunal mucosal culture media, these having been measured simultaneously in the same patients, the hypothesis being that as one decreased the other would increase. The correlation coefficients were therefore calculated for the migration indices for peripheral blood and organ culture media for the untreated coeliac patients ($r = -0{\cdot}79$, $P < 0{\cdot}05$), the treated coeliac patients ($r = -0{\cdot}69$, $P < 0{\cdot}05$) and for the whole group ($r = -0{\cdot}73$, $P < 0{\cdot}005$, Fig. 1). There was a significant negative correlation for each group of patients, indicating that as migration inhibition increased in the peripheral blood it decreased in the mucosal culture medium, and vice versa. The appended numbers in Fig. 1 indicate the degree of strictness of

Table 2. Migration indices from peripheral blood leucocytes and organ culture media in coeliac patients

No.	Peripheral blood leucocytes	Culture medium
Untreated		
1	0·72	1·12
2	0·78	1·13
3	0·91	1·02
4	0·77	1·06
5	0·86	1·02
6	0·73	1·10
7	0·92	0·79
Treated		
1	0·68	1·08
2	0·74	1·01
3	0·76	1·08
4	0·80	1·08
5	0·87	0·95
6	0·78	1·14
7	0·84	0·90
8	0·91	0·93
9	0·77	1·05

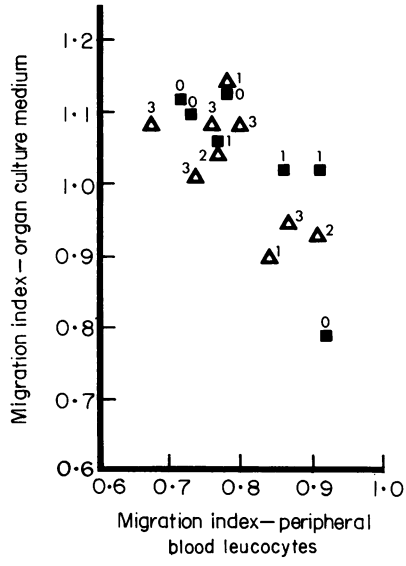

Fig. 1. Migration indices for peripheral blood leucocytes and organ culture medium from coeliac patients, measured simultaneously. Appended numbers refer strictness of gluten-free diet (see text) ($r = -0{\cdot}73$, $P < 0{\cdot}005$). Untreated (■); treated (△).

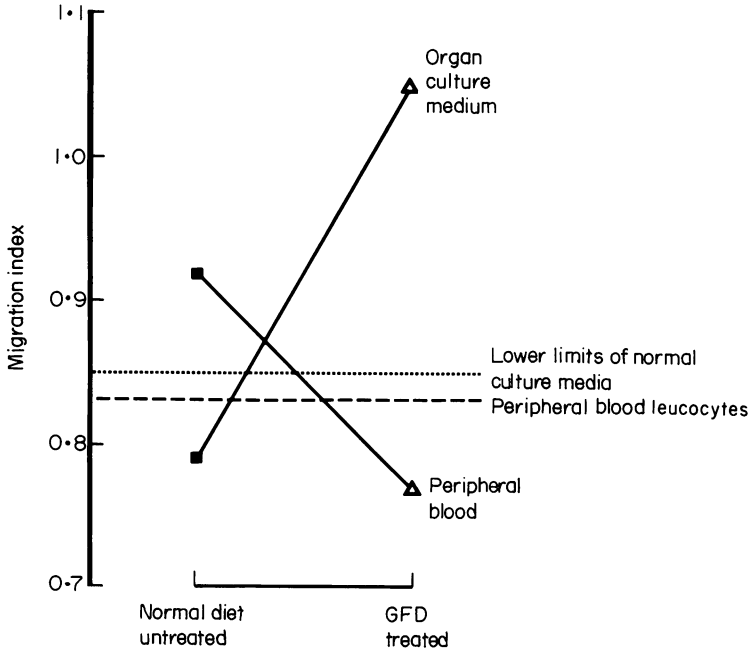

Fig. 2. Migration indices in one coeliac patient for peripheral blood and organ culture media before (■) and after a gluten-free diet (GFD; △).

a gluten-free diet in these patients, but these were randomly distributed. It is important to note that, although four of the untreated patients were taking a normal diet (Grade 0), three had to be classified as taking a poor gluten-free diet (Grade 1) since they were siblings of a known coeliac

patient and it seemed they were already taking a diet low in gluten before presenting for investigation and treatment.

One patient (No. 7, untreated) was also a treated patient 6 months later (No. 9). In her case, the results have been compared before and after treatment, and are plotted in Fig. 2. This Figure also shows the lower limits of the normal ranges of migration indices for normal control patients, as already published (Bullen & Losowsky, 1978; Howdle *et al.*, 1982). These results show that before treatment, in the untreated state on a normal diet, there was significant migration inhibition produced by a factor in the organ culture medium from culture of the untreated jejunal mucosa; simultaneously there was no inhibitory factor produced by the patient's peripheral blood leucocytes. Conversely, after 6 months treatment with a gluten-free diet and an improvement in jejunal morphology to mild partial villous atrophy, the mucosa then produced no inhibitory factor into the culture medium but there was such a factor produced in the peripheral blood, causing significant inhibition of migration of leucocytes.

DISCUSSION

These results show that simultaneously obtained migration indices from jejunal mucosa and peripheral blood were significantly negatively correlated in these coeliac patients. This was so with the untreated and treated groups separately, and with both grouped together. These findings suggest that if a migration inhibition factor is detected from the mucosa, it is less likely to be detected in the peripheral blood, and vice versa. This lends support to the suggestion of Holmes *et al.* (1976) that gluten-sensitive lymphocytes may be sequestered in the gut at one time and be predominantly circulating at another. In one patient, who was studied before and after treatment, this type of lymphocyte distribution was clearly related to the gluten content of the diet.

Comparisons between patients, however, did not reveal a relationship between the state of treatment and mucosal or systemic CMI. This may be purely fortuitous but is not unexpected since in our previous studies of migration inhibition in coeliac disease there was a major overlap in results between treated and untreated patients for migration indices from peripheral blood leucocytes (Bullen & Losowsky, 1978) or jejunal organ culture media (Howdle *et al.*, 1982). The lack of relationship with diet may also be compounded by such factors as variation in the degree of immune response between patients or over time (Simpson *et al.*, 1981), the relatively small number of patients studied, the fact that three of the untreated patients were partially treated in dietary terms, and the relatively imprecise assessment of the degree of strictness of adherence to a gluten-free diet where perhaps a quantification of the gluten content of individual diets might have revealed a significant relationship. Nevertheless there is no doubt that the migration indices showed a significant negative correlation, supporting the type of distribution of lymphocytes suggested above.

It has to be recognized that, although the migration inhibition test is thought to represent a cell-mediated immune reaction, that is, a function of T lymphocytes, other explanations are possible. Migration inhibition can be mediated by cytophilic antibody (Brostoff, 1974; Ortiz-Ortiz *et al.*, 1974) and there is little doubt that coeliac jejunal biopsies during organ culture produce increased amounts of immunoglobulin (Wood *et al.*, 1986) with an increased proportion of antigluten antibody (Ciclitira *et al.*, 1986). We have also produced evidence that coeliac serum contains cytophilic antibody which causes migration inhibition of normal leucocytes (Simpson *et al.*, 1983). However, whatever the immunological basis of migration inhibition, we have shown that there are inhibitory factors produced in a reciprocal manner by coeliac mucosa or peripheral blood. These findings support the hypothesis that coeliac lymphocytes may be distributed systemically or sequestered within the mucosa, but further work is needed to demonstrate clearly whether this distribution is affected by the presence or absence of dietary gluten.

REFERENCES

BROSTOFF, J. (1974) Critique of present in vitro methods for the detection of cell-mediated immunity. *Proc. Roy. Soc. Med.* **67,** 514.

BULLEN, A.W., LOSOWSKY, M.S. (1978) Cell-mediated immunity to gluten fraction III in adult coeliac disease. *Gut* **19,** 126.

CICLITIRA, P.J., ELLIS, H.J., WOOD, G.M., HOWDLE, P.D. & LOSOWSKY, M.S. (1986) Secretion of gliadin antibody by coeliac jejunal mucosal biopsies cultured in vitro. *Clin. exp. Immunol.* **64,** 119.

FERGUSON, A. (1974) Lymphocytes in coeliac disease. In *Coeliac Disease* (ed. by W.Th.J.M. Hekkens & A.S. Pena) p. 265. Stenfert Kroese BV, Leiden.

FERGUSON, A., MACDONALD, T.T., MCCLURE, J.P. & HOLDEN, R.J. (1975) Cell mediated immunity to gliadin within the small intestinal mucosa in coeliac disease. *Lancet* **i,** 895.

FRAZER, A.C., FLETCHER, R.F., ROSS, C.A.C., SHAW, B., SAMMONS, H.G. & SCHNEIDER, R. (1959) Gluten-induced enteropathy. The effect of partially digested gluten. *Lancet* **ii,** 252.

HOLMES, G.K.T., ASQUITH, P. & COOKE, W.T. (1976) Cell-mediated immunity to gluten fraction III in adult coeliac disease. *Clin. exp. Immunol.* **24,** 259.

HOWDLE, P.D., BULLEN, A.W. & LOSOWSKY, M.S. (1982) Cell-mediated immunity to gluten within the small intestinal mucosa in coeliac disease. *Gut* **23,** 115.

HOWDLE, P.D., CORAZZA, G.R., BULLEN, A.W. & LOSOWSKY, M.S. (1981) Gluten sensitivity of small intestinal mucosa in vitro: quantitative assessment of histologic change. *Gastroenterology* **80,** 442.

ORTIZ-ORTIZ, L., ZAMACONA, G., GARMILLA, C. & ARELLANO, M.T. (1974) Migration inhibition test in leukocytes from patients allergic to penicillin. *J. Immunol.* **113,** 993.

SIMPSON, F.G., BULLEN, A.W., ROBERTSON, D.A.F. & LOSOWSKY, M.S. (1981) HLA-B8 and cellular immune response to gluten. *Gut* **22,** 633.

SIMPSON, F.G., FIELD, H.P., HOWDLE, P.D., ROBERTSON, D.A.F. & LOSOWSKY, M.S. (1983) Leucocyte migration inhibition test in coeliac disease—a reappraisal. *Gut* **24,** 311.

WOOD, G.M., SHIRES, S., HOWDLE, P.D. & LOSOWSKY, M.S. (1986) Immunoglobulin production by coeliac biopsies in organ culture. *Gut* (in press).